Chapter 6

Immune System

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The overall goal of space immunology is to clarify how weightlessness affects immune-system responses in humans, with particular attention given to lymphocytic homeostasis, stability of antigenic structures, and genetic stability of somatic cells. The immune system plays a crucial role in preventing the development of various pathologies. Any reduction in the ability of immunocompetent cells to combat foreign structures leads to secondary immunodeficiency and poses greater risk for disease development, including bacterial and viral autoinfections. Autoimmunity, in which the immunocompetent cells of the body react to its own antigens as foreign, may also occur. Unusually strong immune responses to external antigens may cause allergies.

Spaceflight conditions expose the human immune system to many potentially adverse factors, including confinement in a crowded, closed environment and hypokinesia within that environment. Psychological stresses also are known to trigger changes in the functional complex of the cerebral cortex, hypothalamus, pituitary, adrenal, and thymus glands. A classic stress response, involution of lymphoid organs, was noted in animals flown on Kosmos biosatellites.[1] Animal studies can be supplemented with assessments of isolated immunocompetent cells and ground-based flight simulations with humans to clarify the mechanisms underlying human responses to actual spaceflight conditions.

Another objective of space immunology is to establish a relationship between immune-system impairment during flight and the status of other physiological systems. Recent seminal investigations have linked several immune mediators with regulation of skeletal calcium metabolism; spaceflight simulations have established a correlation between the function of human immunocompetent cells and regulation of osteoclasts, and have raised several new theoretical and practical issues. The intent of this chapter is to provide a summary of experimental results of these and other investigations conducted by the Russian and U.S. space programs.

I. Spaceflight and the Human Immune System

Any comprehensive discussion of immune function during spaceflight should address the following issues:

- What exactly are the effects of spaceflight on the human immune system?
- How do changes in immune-system responsiveness affect crew member health during and after flight?
- Are the nature and severity of observed changes in the immune system linked to the duration of spaceflight?
- How do weightlessness, stress, and other spaceflight factors contribute to these changes?

Studies of immune function in crew members who have flown on the Soyuz and Space Shuttle spacecraft and the Skylab, Salyut-6 and -7, and Mir space stations have generated extensive results over the past 15 years. These studies, to a large extent, were based on observations from the earlier Apollo[2] and Soyuz[3,4] missions. Many of the missions flown were unique in terms of their duration, the amount and nature of work performed by the crew members, and the use—or lack thereof—of countermeasures. Individual differences in immune reactivity undoubtedly contributed to variation between flights as well. Nonetheless, some general conclusions can be drawn from the results obtained from these studies.

A. Lymphocyte Function (Preflight and Postflight)

The ability of a mitogen (e.g., phytohemagglutinin, or PHA) to induce lymphocyte activity in vitro is used widely in clinical immunology as a general indicator of immune status. The proliferation of lymphocytes in response to a mitogen reflects a sequence of processes that define the normal response of this cell population to an external stimulus (including microbial and viral invasion). This process includes the activation of thymus-dependent (T) cells; the production of humoral mediators activated by the T cells [e.g., interleukins (IL-2, IL-1, and so on), interferon-γ (IFN-γ) and others]; the expression of surface receptors to IL-2; and finally lymphocyte proliferation.

T-cell response to PHA has been studied in space crews before and after flight, with either radiometry (U.S.) or radioautography (U.S.S.R.) being used to estimate the synthesis of RNA and DNA through uptake of radiolabeled ^{3}H-uridine and ^{3}H-thymidine, respectively. Spaceflight-induced immunosuppression was first revealed with this technique after the Soyuz-6, -7, and -8 missions.[3,4] Mononuclear cells stimulated by PHA assessed from 21 Apollo astronauts before and after flight revealed no significant abnormalities after flight.[2] However, PHA responsiveness was reduced after all three Skylab missions,[5]

with reductions in both RNA and DNA synthesis revealed after the two longest flights (59 and 84 days). Interestingly, decrements in these indicators were noted in blood samples taken from the Skylab-3 crew immediately before launch and in samples from the Skylab-4 crew 30 and 7 days before launch. Other decrements in immune function have been observed during the immediate preflight period in other programs as well.[6-8] Immune suppression during this period, as compared to a baseline established by numerous earlier tests, has been attributed to the physical, psychological, and emotional stresses that crews undergo during the preflight period. Berry[9] has often underscored the clinical consequences of excessive preflight astronaut training.

Blastogenesis in response to both mitogen and flu-virus antigen was suppressed after the Apollo-Soyuz mission[10]; however, this crew had been exposed to the toxic irritant N_2O_4 during landing. Later assessments of lymphocyte responsiveness after Space Shuttle flights[11,12] have benefitted from improvements in measurement techniques. In one such study of 36 crew members from 11 missions, the T-cell response to a specified amount of PHA was diminished after the first four 2- to 8-day missions (by 18% to 61% of baseline). Significant reduction in ^{3}H-thymidine uptake was found for 29 astronauts, but no suppression of PHA response was noted in five others. These investigators believe that the severity of the attenuated PHA responsiveness is correlated with the magnitude of in-flight stress. Mononuclear cell-surface phenotype assessed in 11 Space Shuttle crew members revealed a positive correlation between reduced ^{3}H-thymidine uptake in PHA-stimulated cells and the $M3^+$ cell population, but not with $CD19^+$ (B), $CD4^+$ (T-helper), or $CD8^+$ (T-suppressor) cells.[13]

The more interesting of the Soviet studies of more than 80 cosmonauts are those reflecting the effects of several-month stays. Most of the cosmonauts who lived on board the Salyut-6 and -7 and Mir stations for 2 to 12 months showed substantial declines in PHA response by T cells on the first day after landing (Fig. 1), particularly with regard to RNA synthesis. By the seventh day after landing, the T-cell response to PHA had returned to normal for many, and was below normal on day 30 for only two cosmonauts. ^{3}H-thymidine uptake was below normal in only one-third of this group after flight, suggesting that the RNA-synthesis test is the more sensitive of the two. With regard to mission duration, six of the 29 cosmonauts who flew 6- to 10-day missions on Salyut-6, Salyut-7, and Mir showed a decline in PHA responsiveness. However, this decrease was not significant in most cases, with the average value remaining within the norm (18.9 ±1.44%). Only one cosmonaut showed a marked suppression of this lymphocyte function. After landing, this same cosmonaut showed decreases in both RNA-synthesis rate and number of radiolabeled cells. No radiolabeled ^{3}H-uridine cells were found on the first day after landing, and only 8.6% were present by the seventh day (normal range is 15 to 26%). The proportion of ^{3}H-thymidine-labeled lymphocytes in this same cosmonaut was 5.4% on postflight day 1 and 3.5% on postflight day 7 (normal range is 16 to 40%). Clinical examination revealed malaise, subfebrile temperature, weakness and perspiration, all of which subsequently disappeared.

T-cell responses to PHA thus seem to differ in cosmonauts who flew for 10 days or less vs those who flew 2- to 12-month missions. Responsiveness to PHA was reduced only after long flights on Salyut-7 and Mir. However, no further decline was noted when flight duration increased from a few months to 12 months. The average value of lymphocyte responsiveness for 16 cosmonauts who flew from 4 to 6 months was 13.4±0.78%, quite similar to that obtained for nine cosmonauts after 7- to 12-month missions (13.7±0.96%) (Fig. 2). However, this measure was no different from preflight measures in three of the 16 who flew 4- to 6-month missions, and three of the nine who flew 7- to 12-month missions. This important finding suggests that some crew members, even after 8 or 10 months in flight, had rather high T-cell responsiveness—at least at the end of the mission.

In other investigations, T-helper activity was assessed using a technique developed in the author's laboratory based on a xenogeneic "graft-vs-host" response (GVH).[6,14] The mean GVH response was diminished during the early postflight period in almost all crew members who flew long missions ($p<0.05$). Seven days after landing, the group mean returned to normal. By the 45th day after landing, the mean T-helper value had increased; however, T-helper activity did not decrease after short (6- to 10-day) flights (Fig. 3).

In vitro activity of another T-cell population, the suppressor T cells, induced by concanavalin A (Con A), another mitogen, remained unchanged in half of the crew members after long missions.[6] Some crew members had increases in suppressor T-cell activity during the first seven days after landing, followed by recovery; others, including the two crew members who completed a 150-day mission on Salyut-6, showed the opposite effect. This result could reflect possible toxic effects of airborne contaminants found during flight. However, an analogous decrease in suppressor-T activity was observed in some other cosmonauts. Nonspecific suppressor activity was virtually unchanged after brief flights. Cytofluorimetric analysis revealed decreases in the $CD4^+/CD8^+$ ratio in some cosmonauts because of a decreased number of $CD4^+$ cells. Clinically, a ratio of this kind is viewed as resulting from secondary T-cell insufficiency.

The activity of another type of lymphocyte, natural killer (NK) cells, also is affected by spaceflight. NK cells normally protect hosts against tumor cells or cells infected by viruses as well as mediating GVH disease and other functions. More than 80% of crew members had suppressed NK-cell cytotoxic activity after long missions on Salyut-6, Salyut-7, and Mir[6,8] (Fig. 4). After 7- to 7.5-month missions on Salyut-7, the greatest suppression of NK-cell activity was noted the first day after landing, after which NK-cell cytotoxicity returned to preflight levels (Fig. 5). The dynamics of NK-cell activity after later missions on Mir (lasting 4 to 6 months) were consistent with previous results. However, of the four cosmonauts who flew 8-, 11-, or 12-month missions, NK-cell cytotoxicity was consistently high at all measurement periods. No significant

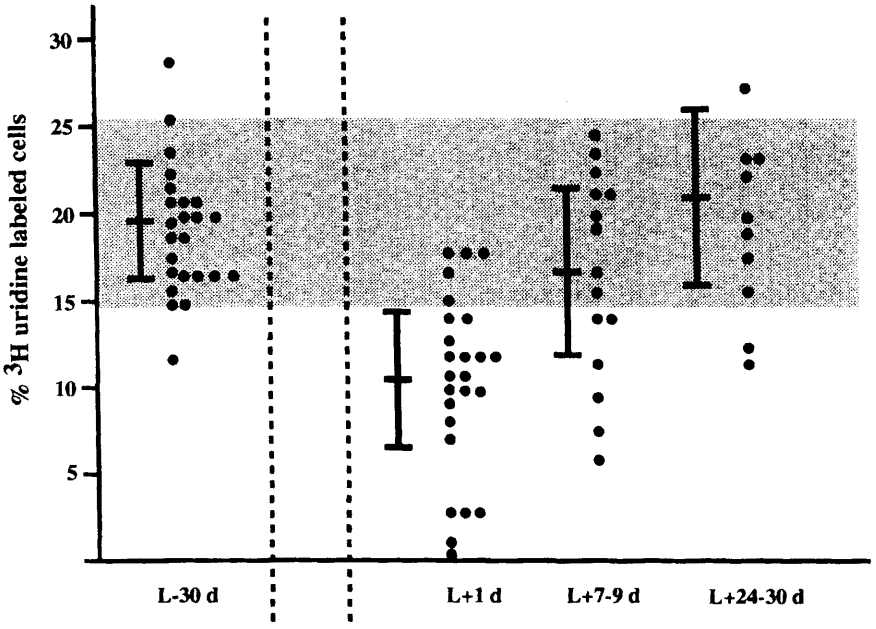

Fig. 1 PHA response of T-lymphocytes in cosmonauts after flights on Salyut-6 and -7, 65–237 days in duration. The shaded area is the range of normal variation.

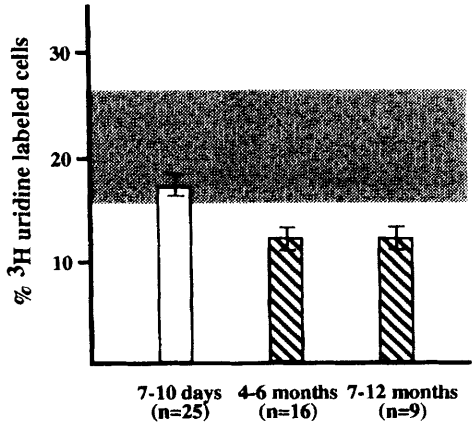

Fig. 2 PHA response of T-lymphocytes in cosmonauts after missions of 7–10 days, 4–6 months, or 7–12 months. The shaded area is the range of normal variation.

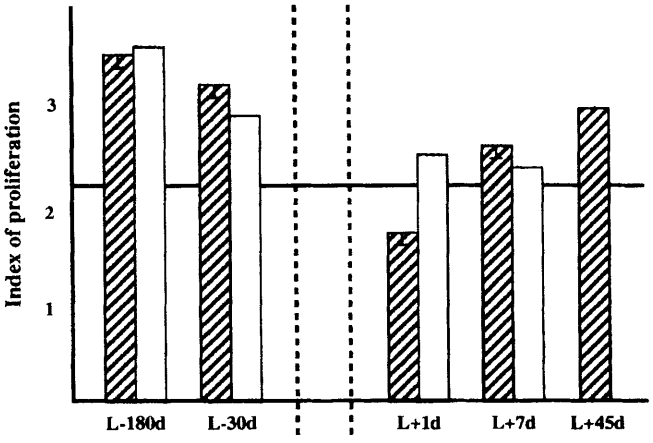

Fig. 3 T-helper-cell activity in cosmonauts before and after either long missions (75–237 days, shaded bars) or short missions (6–10 days, unshaded bars) on Salyut-6 and -7. The line represents the lower boundary of normal.

changes were present in any other immune-function indicator measured. This discrepancy between mid- and long-duration flight certainly could have reflected individual differences, or differences in the countermeasures that were used.[16] Nonetheless, these results suggest that the immune system (or at least some of its components) could be adapting to prolonged exposure to microgravity.

NK-cell activity was also examined before and after Salyut-7 and Mir flights at the level of single cells. Tests in thin layers of agar revealed diminished capacity of lymphoctyes to bind target cells after long missions. The proportion of lymphocytes that bound K-562 cells before flight was 16.9±2.3%; this number dropped to 3.3±0.4% on the first day after landing, and had increased to only 8.2±1.03% six days later. These changes could have resulted either from an insufficient number of mature NK cells entering the blood from the bone marrow, or from suppressed expression of specific cell surface receptors. Interestingly, lysis of target lymphocytes in conjugates was not diminished.[15,17]

Scanning electron microscopy of NK cells bound to target cells revealed unusual ultrastructure changes in the secretory and locomotor apparati of the NK cells. Some cells were bound to the targets but did not have activated secretory apparati; others had activated secretory apparati but the Golgi complexes and secretory granules were not oriented toward target cells. Still others had hypertrophic Golgi complexes

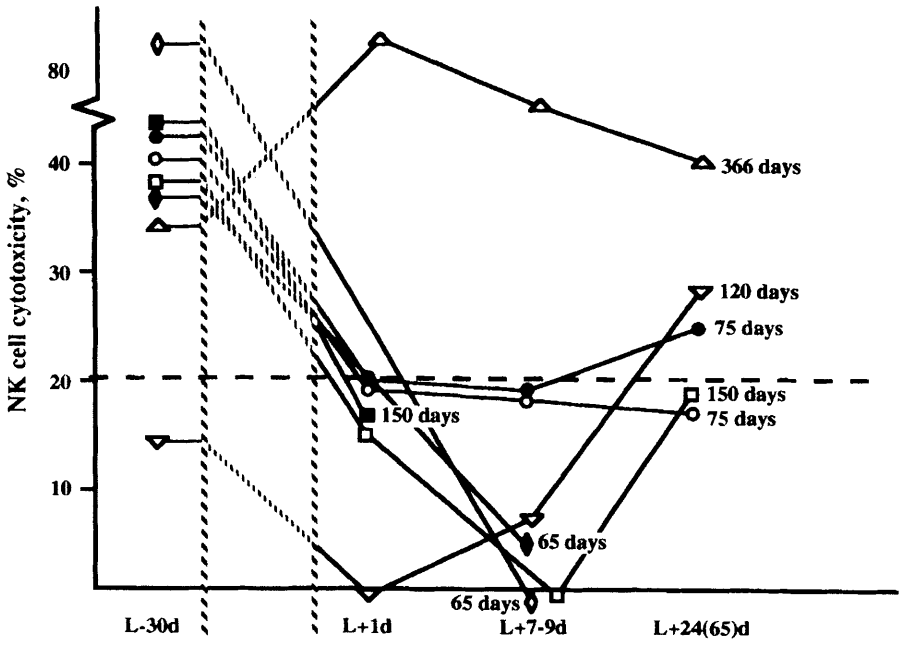

Fig. 4 Natural-killer-cell activity in eight cosmonauts after flights of various durations on Salyut-6, Salyut-7, and Mir. The dashed line at 20% is the lower boundary of normal.

but no secretory granules or microtubules, and some had "new" fibrillar, crystalline, or tubular structures that interfered with orientation of the secretory apparatus. Similar results have been found after head-down bed rest.[6]

NK-cell cytotoxicity frequently was reduced on the first day after even 6- to 8-day missions, suggesting that stress induced by acute adaptation to weightlessness may have been responsible. Stress hormones, particularly glucocorticoids, cause rapid declines in NK-cell activity in vitro.[18,19] The underlying cause for changes in NK-cell activity during or after long missions is unknown. However, observations of increased frequency of acute respiratory infection, tonsillitis, and other illnesses immediately after flight underscore the importance of investigating countermeasures for weakened antiviral resistance associated with spaceflight.

B. Cytokine Production (Preflight and Postflight)

Activation of T cells is the basis for the defense mechanisms of the immune system. This activation process is mediated by at least 90 cytokines, regulatory proteins secreted by lymphocytes and monocytes. Activated T cells also generate cytokines, which trigger a cascade of interrelated reactions.

Interleukins are cytokines that regulate the interactions between lymphocytes and other leukocytes. Interleukin-2 (IL-2) production by lymphocytes, and expression of T-cell-activated IL-2 receptors, were measured after brief missions (lasting 2 to 10 days) and after long missions (lasting 63 to 366 days) on Salyut-7 and Mir.[20] The CTLL cell line, 7-day-old human lymphoblasts, and depleted 10-day-old unstimulated human lymphocytes were used to test IL-2 production in vitro in response to PHA.[20] IL-2 also was quantified by enzyme-linked immunosorbent assay (ELISA) using monoclonal antibodies to recombinant IL-2.[21,22]

Biological activity of IL-2 was reduced in 17 of the 19 cosmonauts examined. Suppression of IL-2 at landing was correlated with mission duration, with the greatest suppression (relative to preflight activity) after the longest flights (Fig. 6). By the seventh day after landing, the mean IL-2 activity had increased slightly but was still well below preflight activity. IL-2 activity also was suppressed after shorter missions, but to a much lesser extent. In contrast to these results, the ELISA technique indicated that IL-2 production was not suppressed relative to preflight baselines regardless of flight duration, and in some cases increased over preflight values. IL-1 production in some cosmonauts either remained unchanged or increased. Lymphocyte-activated expression of cell receptors to IL-2, measured by cytofluorimetry, was unchanged after landing.[22] These data suggest that secretion of some molecular substances may be enhanced in flight, possibly as a compensatory response to depression of other immune elements.

Production of endogenous IFN-α in vitro in response to lymphocyte stimulation by Newcastle virus was tested before Salyut-6, Salyut-7, and Mir as well. Postflight measurements were equivocal, with no change from preflight noted for some, increases in others, and decreases in still others. Of the eight cosmonauts tested after flights on Mir, only two showed a decline in IFN-α production after flight, and IFN-γ production by PHA-stimulated lymphocytes was reduced in some but not all cosmonauts.

Osteoclast-activating factor (OAF), produced by mononuclear cells, is known to be produced in greater amounts in the presence of elevated calcium loss from bone. Three of eight cosmonauts tested after Salyut and Mir missions had

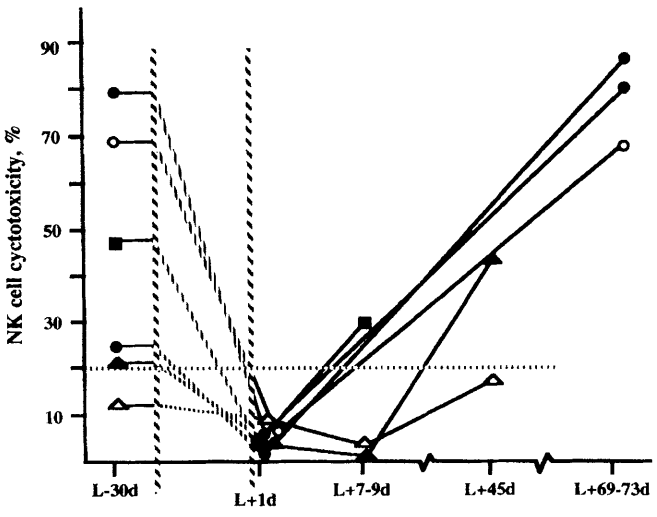

Fig. 5 Natural-killer-cell activity in six cosmonauts after 211- to 237-day missions on Salyut-7. The dashed line at 20% is the lower boundary of normal.

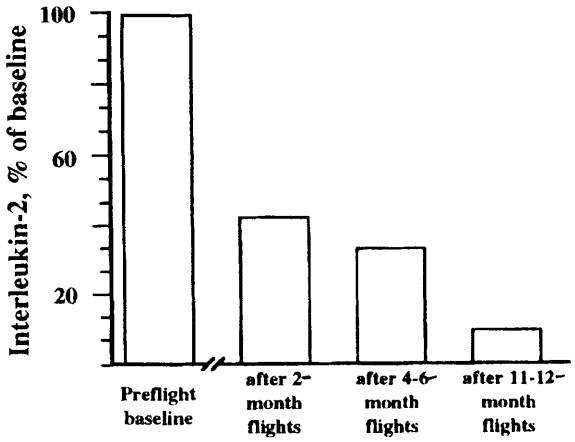

Fig. 6 Interleukin-2 production by cosmonaut lymphocytes in response to PHA on landing day after flights of various durations.

significantly greater production of OAF after flight than before. This finding did not seem to reflect mission duration, as one flew 8 days, the second 65 days, and the third 327 days. These intriguing results underscore the need for further exploration of the idea that countermeasures against calcium loss in weightlessness could be aimed at the immune regulatory mechanisms underlying this process.

Postflight proportions of immunoglobulins (Ig) in blood were no different in most crew members after flight than before, although a brief, reversible increase in IgA or IgG[2,6] was noted in some cases. Tests of autoimmunity, e.g., response to rheumatoid factor (RF) or antibody production in response to single-strand DNA, revealed negative results both before and after flight, except for one cosmonaut. That individual, who had a distinctly positive reaction (+++) to RF on the eighth day after a long mission, also had acute nephritis, and his response was thought to reflect a preexisting compensated autoimmune process.[6] This cosmonaut also displayed microhematuria at several measurement sessions.

Hypersensitivity to allergens was present after landing in four cosmonauts *(Streptococcus* allergens in three and formaldehyde in the fourth subject).[6,23] Activation of preexisting hypersensitivity to bacterial allergens was demonstrated during flight in two other crew members using in vitro tests of specific lymphocyte blast transformation and inhibition of specific leukocyte migration. Sensitivity to chemical allergens (formaldehyde and other substances) was detected with a skin test and leukocyte agglutination. Although these data are extremely limited, the risk of allergic reaction logically would increase with flight duration. Aside from the issue of immune-system impairment, the risk of allergic reactions increases with the length of time humans are aboard the station, since this increases both the amount and the range of atmospheric contaminants that can act as allergens. Microbial contamination on board also needs to be considered, and remediation applied as needed.

C. Humoral and Cellular Immunity (During Flight)

The first studies of immunoglobulin (antibody) concentrations during spaceflight were Kimzey's assessments of IgA, IgM, and IgG in blood samples from the Skylab crews, who collected samples from each other during flight and stored them in the on-board freezer for later return to Earth. All Ig levels assessed were within normal limits.[5] Later Soviet studies made use of an in-flight device designed to allow blood to be taken from finger-sticks and diffused into a gel substrate.[6] Between 1980 and 1992, blood antibody levels were measured in 17 Soviet and Russian cosmonauts during long-term flights. One of the two cosmonauts studied on flight day 13 showed a rise in IgA; on flight days 52, 104, and 192, IgM was elevated considerably (up to 340 mg %) in most cosmonauts. Elevated IgM levels usually are associated with increased microbial contamination of the station.

A classic test of cell-mediated response to antigens is to administer an antigen subcutaneously to an individual who has already been exposed to that antigen. Because any reaction to the antigen typically takes 24 hours to develop, this test describes delayed-type hypersensitivity (DTH). DTH tests became standard means of assessing cellular immunity during both Space Shuttle and Mir missions. The equipment used in both programs, parts of which were designed jointly by Soviet and Swiss engineers specifically for space applications, was slightly different but worked on the same principle. The test device is a slightly modified version of a Merieux multitest device that contains seven standard antigens, five bacterial *(Streptococcus, Clostridium tetanus, Proteus, Bacillus diphtheria, Bacillus tuberculosis)* and two fungal *(Trychophyton and Candida),* normal variance of which has been established for adult men. Correlations have been established between

decline in the DTH reaction (as measured by the Merieux multitest) and immune deficiency.

DTH reactions were studied in a Soviet-Swiss experiment with six cosmonauts on four Mir missions; the missions lasted 132, 145, 177, or 312 days. Measurements were made 2 to 12 months before launch, between 59 and 240 days of flight, and on the eighth day after landing (three cosmonauts were tested twice during flight and the others were tested once). Two cosmonauts had significantly decreased DTH reactions twice, one on flight days 59 and 155 and the other on flight days 110 and 240. A third cosmonaut showed a decrease on flight day 110. The remaining three cosmonauts had no appreciable changes in DTH during flight, although one may have tended toward suppression of the DTH reaction. One cosmonaut's results were normal on flight day 59, had decreased to the lower limit of normal on flight day 155, and had increased to normal (27 mm) by the eighth day after landing. A control group of 14 healthy men maintained normal responses throughout the test period. Interestingly, attenuated reactions to the skin test were noted for some cosmonauts after particularly difficult assignments such as extravehicular activities.

More recent results suggest that the severity of immune-system dysfunctions may be a function of flight duration, at least when flights of 7 to 9 days are compared to those lasting months. However, no reliable differences in deterioration were noted for flight durations ranging from 2 to 12 months.[24] Moreover, crew members from the longest mission (12 months) showed less severe dysfunction, both in the immune system and other physiological systems, than did crew members from shorter missions. Future studies are needed to assess the significance of individual differences, compliance with countermeasures during flight, and other factors.

II. Human Immune Function in Ground-Based Simulations

A. Head-Down Bed Rest

Bed rest with head-down tilt, used to model weightlessness, has been used to study immune function in more than 60 subjects[6,23] confined for 8, 35, 120, or 182 days. Immune system status was assessed in these subjects before, during, and after the bed-rest period. Bed rest did not change T and B cell counts in blood. The in vitro T-cell response to PHA was reduced in 15 of the 18 subjects in the 182-day study, but the reductions were both moderate and transient. Similar data were obtained in other long-term experiments. NK-cell cytotoxicity either remained constant or was reduced at certain points during bed rest.[25] However, subjects in the former group showed sharp declines in NK-cell cytotoxicity during the first week after bed rest, while subjects in the latter group returned quickly to normal levels during that time (Fig. 7). Since experimental conditions were the same for all subjects, this observation underscores the importance of individual differences in responsiveness.

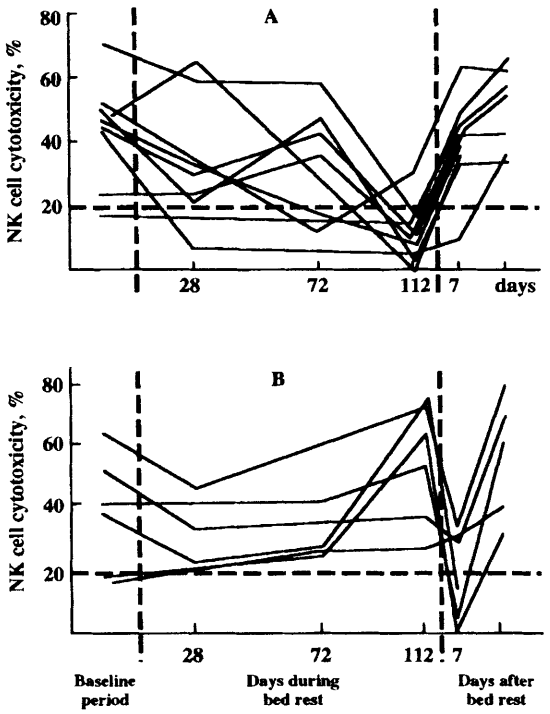

Fig. 7 NK-cell cytoxicity before, during, and after 120 days of bed rest. Cytotoxicity in nine subjects (top panel, A) was diminished during bed rest but recovered quickly. Cytotoxicity in another six subjects (bottom panel, B) remained fairly constant during bed rest, but had dropped sharply at 7 days afterward.

T-helper activity, as evaluated by graft-vs-host reactions, was generally unchanged, decreasing in some subjects only occasionally. Nonspecific suppressor T-cell activity also remained the same or occasionally increased. Notably, some subjects became ill during hypokinesia: Of 18 subjects, six developed furunculosis at different periods during the 182-day study, 10 had acute respiratory tract or adenoviral infections, five had periodontitis, and five developed dermatitis of unknown etiology. These disorders tended to coincide with a decline in PHA responsiveness.

Because prolonged bed rest in clinical situations has been associated with allergic responses,[26] sensitivity tests were given to the head-down bed rest subjects as well, including tests of specific leukocyte blast transformation in response to bacterial allergens; leukocyte-inhibiting factor (LIF) production; and skin sensitivity. The bacterial allergens tested were *Staphylococcus, Streptococcus,* and *Escherichia coli.* Of the 18 subjects in the 182-day study, seven developed sensitivity to some bacterial allergens, usually within 45 to 90 days of hypokinesia. Four subjects showed increases in the spectrum of microbial allergens that produced delayed skin reactions or lymphocyte blast transformation. The frequency and duration of acute respiratory tract and adenoviral infections, periodontitis, and dermatitis was approximately the same in all subjects regardless of their sensitivity. However, the average duration of

furunculosis in subjects who developed sensitivity to bacterial allergens of a single staphylococcal strain, or to several allergens, was much longer (17.2 days, which is 2.8 times higher than the average duration in the group that did not develop sensitivity). These studies revealed close correlations between the onset of hypersensitivity to staphylococcus antigen and predisposition to furunculosis. These changes also correlated with the density of the staphylococcal flora in mucous membranes. Head-down bed rest also seems to suppress the DTH reaction. Of the 12 subjects tested, four showed no change, and six displayed reactions that were somewhat below their baseline measures.

These studies indicate an association between hypersensitivity to allergens and prolonged hypokinesia, which carries important implications for general as well as space medicine. These observations also confirm our early predictions of allergic reactions in spaceflight.[27]

B. Closed Environments

Another simulation of spaceflight conditions involves the use of artificial atmospheres in an enclosed environment. When such habitats were maintained within sanitary norms, no effects on the immune system were observed.[28] However, another study revealed that changing aspects of that environment (e.g., temperature, humidity, amounts of airborne contaminants) could induce immune-system changes in healthy humans. When the air-purification system was periodically shut off in one such environment, atmospheric contaminants accumulated quickly; although the PHA responsiveness of T cells did not change, the numbers of B cells increased markedly, and IgG and IgA concentrations increased. Immune-system dysfunction was positively correlated with prolonged exposure to high temperatures (to 33°C) as well, which promote increases in contaminants. Three subjects underwent a 90-day test in a closed environment in which carbon monoxide concentration was maintained at 15 mg/m^3, with two brief increases to 45 mg/m^3 (Fig. 8). ^{3}H-uridine uptake by PHA-stimulated lymphocytes from these subjects fell in response to the increase in CO concentration, and were still well below the normal range on the eighth day after the confinement had ended.[28]

In another study, hypercapnic conditions in a closed environment (1.5% CO_2, with periodic increases to 4%) led to significant declines in NK-cell activity and the ability of effector cells to bind target cells. These changes were accompanied by an increase in IgG titer, but PHA responsiveness was unchanged. The sensitivity of NK cells to excessive CO_2 has been confirmed in other studies. Acetic acid, which is released as a metabolic waste product by humans, was maintained at a starting concentration of 5 mg/m^3 and increased to 10 mg/m^3 over a 15-day period. T-cell responsiveness to PHA in this environment decreased slightly, and NK-cell cytotoxicity declined significantly, when acetic acid concentrations reached 10 mg/m^3. Neither the activities of T-helper or T-suppressor cells nor the titers of IgA, IgG, or IgM changed in this study.

These findings suggest that some forms of atmospheric contamination in closed environments can produce changes in some immunological factors. NK-cell cytotoxicity and ability to recognize and bind target cells proved to be the most unstable during prolonged exposure of healthy humans to high CO_2 concentrations. Analogous deviations, though less pronounced, have been found in cosmonauts. These effects could be related to diminished production and migration of NK cells from the bone marrow, or perhaps to direct or indirect effects of spaceflight factors on NK-cell neurohormonal regulatory systems. Some evidence exists to suggest that glucocorticoids can suppress NK cells,[18] but neither migration, recognition, nor lysis were tested in that investigation. On the one hand, glucocorticoids seem to induce synthesis of lipomodulin, a phospholipase A_2 inhibitor that obstructs the early phase of lymphocyte activation.[19,29] On the other hand, oxidation products of arachidonic acid in general—and prostaglandin E_2 in particular—are known to inhibit NK cytotoxicity in humans, suppressing (through adenylate cyclase and cAMP) the ability of NK cells to recognize and bind target cells.[30] It is still not known whether this suppression is associated with diminished synthesis and expression of receptors of NK-endogenous lectin or impairment of the NK-cell locomotion function. However, our findings that exposure to 4% CO_2 in a closed environment resulted in diminishment of NK-cell activity and number of conjugates, while NK-cell cytotoxicity was preserved, bear directly on this issue.

C. Stress Models

Distinguishing between the effects of weightlessness per se and the effects of stress on the immune system poses considerable challenges for seeking the origin of immune dysfunctions during flight. We believe that focusing on neurohormonal mechanisms can shed light on questions regarding immunologic aspects of extreme environments. Research in this area began in the early 1950s, when relationships were first established between psychological stress and sensitivity to bacterial and viral infections and cancer. Later, thymocytes and other immune cells were found to have surface receptors having affinity for hormones and peptides. Immunocompetent cells were shown to have receptors for stress mediators (catecholamines, corticosteroids, and acetylcholine) as well as for endorphins, serotonin, and substance P. At this point, the importance of identifying stress-induced alterations in the immune system became clear, particularly for involution of the thymus.

At present, two pathways are known by which the immune system is regulated by the nervous system (Fig. 9). The first pathway involves direct communications between the two systems via nerve fibers and endings innervating the thymus, spleen, lymph nodes, and bone marrow.[31] Here, immune cells could be affected through neuromediators such as norepinephrine and serotonin, which already have been shown to affect leukocytes with specific receptors.[32] The second pathway of immunoregulation is humoral, involving pituitary polypeptides

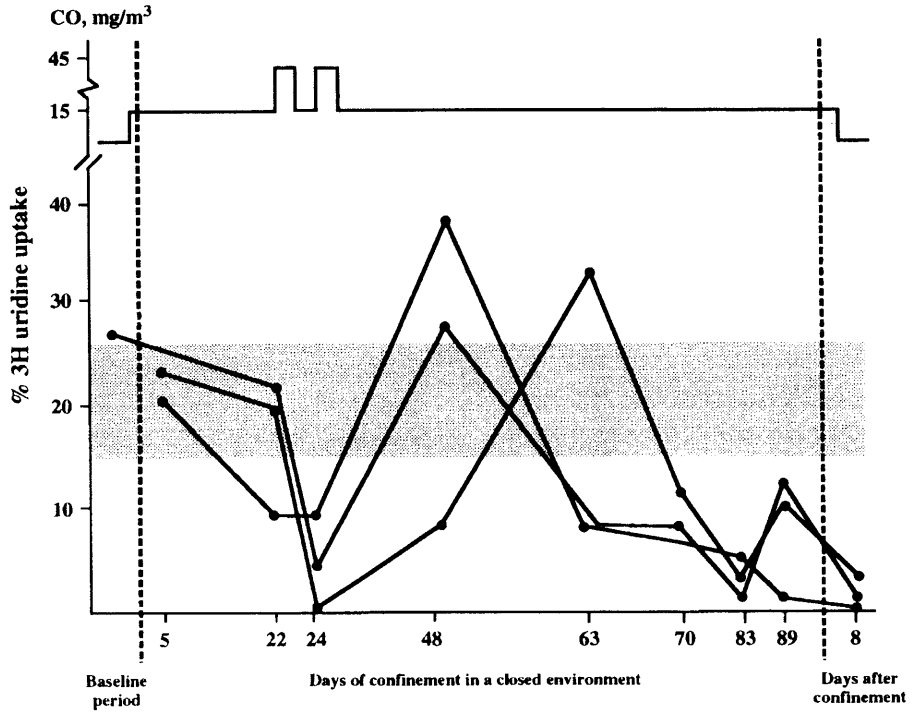

Fig. 8 PHA response of lymphocytes from three subjects confined in a closed environment with 15 mg/m³ carbon monoxide (CO) for 89 days. The line at the top denotes two points at which CO concentrations were increased to 45 mg/m³. The shaded area is the range of normal variation.

and adrenal steroids. Stress is known to elevate concentrations of the latter, which leads to suppression of some immune responses. However, blocking steroid action in rats did not completely eliminate suppression of the immune system,[33] and thus this cannot be the only pathway. In contrast, the endorphin antagonist naltrexone can block stress-induced immunosuppression.[34] ACTH (adrenocorticotropin) and β-endorphin can suppress antibody production in vitro.[32] The list of hormonal factors and hormones that can affect lymphocytes directly through specific receptors is expanding rapidly.

Lymphocytes contain surface receptors for insulin and human growth hormone (HGH).[32,35] Adding insulin to a mixed lymphocyte culture enhances blastogenesis, whereas adding HGH facilitates cytotoxic T-cell production. Vasopressin and oxytocin can function like IL-2 in T-cell-stimulated IFN synthesis. Thyrotropin promotes the generation of antibodies in vitro.[32] Somatotropin, found in mononuclear cells, blocks the release of leukotrienes and the antibody response to sheep erythrocytes. Substance P, which is generated by neurons, enhances T-cell proliferation through receptors on the T cells, induces macrophage chemotaxis, and, like endorphins and enkephalin, participates in immune-system regulation.[36] Glucocorticoids also are thought to be involved in regulation of physiological systems.[37] Thus, humoral substances produced by the nervous system clearly affect cells of the immune system.

Stress-induced changes in humoral and cellular immunity in experimental animals and humans have been described at length elsewhere. Stress (e.g., exposing subjects to noxious stimuli such as noise, light, or types of movement) has been found to induce subnormal responses to antigens and mitogens, decrease cell-mediated cytotoxicity and delay hypersensitivity, attenuate the graft-vs-host reaction, and decrease antibody production. The effect(s) of stress on the immune response is related to the nature and strength of the stimulus as well as biological and psychological traits of the individual. Preexisting illnesses can enhance the suppression of lymphocyte responsiveness under stress. For example, depression, which is known to enhance corticosteroid secretion, was associated with attenuated response of lymphocytes to mitogen[38] as well as the development of autoimmune responses (increased levels of antinuclear antibodies[39]) in severely depressed men.

The presence of direct thymus innervation and mediated innervation of the spleen, lymph nodes, and thymus, plus the presence of adrenergic and cholinergic receptors on lymphocyte surfaces, may reflect a direct relationship between the central nervous system and the immune response to stress. However, stress effects are quite complex, since stress can simultaneously trigger several mechanisms that each have different ultimate consequences. Depending on dose, epinephrine can produce either positive or negative effects on NK cells.[40] This finding contradicts earlier concepts that epinephrine had strictly negative consequences through β-adrenergic receptors and adenylate cyclase. However, these data are far from conclusive, since in addition to the effects of stress on NK cells, this hormone also affects many other central and peripheral processes.

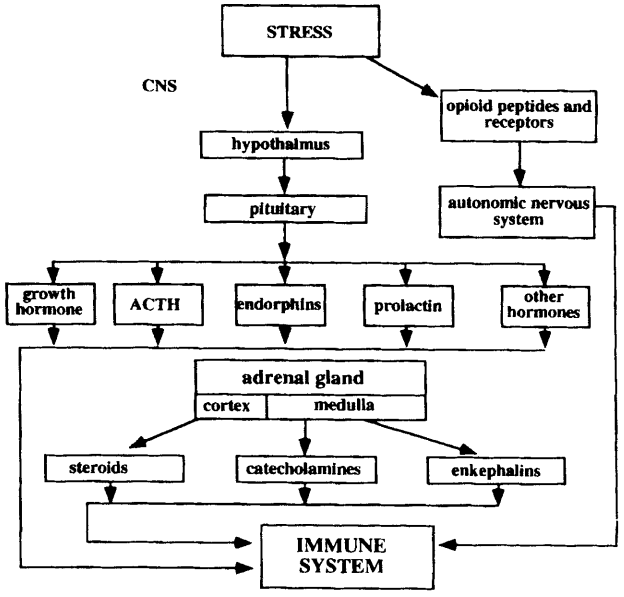

Fig. 9 Neuroendocrine mechanisms of stress effects on the immune system. Reproduced [with permission] from Ref. 35.

The first parachute jump, surely a stress-producing event, led to marked decline in NK-cell activity and production of IFN and IL-1 in healthy adolescents. However, subsequent jumps were not associated with any reduction in NK-cell activity.[41] In another study, Gmunder et al.[42] found that marathon runners had suppressed lymphocytic response to mitogens. Induction of altitude hypoxia in hypobaric chambers,[43] and the psychological stress associated with first-year college examinations, have led to significant reductions in ^{3}H-thymidine uptake by PHA-stimulated lymphocytes, although T cell, B cell, monocyte, NK cell, T helper, and T suppressor counts remained unchanged. Mimicking EVA effects in an altitude chamber did not reveal deviations in cell subpopulations or PHA responsiveness.[43] Combined psychological and hypoxic stress led to diminished uptake of ^{3}H-thymidine by mitogen-stimulated lymphocytes.

Stress effects of spaceflight have been explored by Soviet and Russian investigators for many years.[6,23] Two 60-day clinical experiments repeatedly modeled hazardous and unexpected situations. One situation involved a simulated ascent to 8,000 meters in an altitude chamber; another involved an unexpected announcement of a centrifuge acceleration trial the following day. A third situation involved testing motor coordination and pass-fail logic tasks under time pressure followed by public assessment of the performance of each subject. Ascent in the altitude chamber caused decreased PHA responsiveness of T cells in some but not all subjects. Subjects in the second or third situation showed sharp reductions in T-cell response to PHA. ^{3}H-uridine uptake in those expecting to be centrifuged fell from 16.6 to 2.3% ($p < 0.001$), and in subjects taking the cognitive tests from 19.1 to 6.2% ($p < 0.05$).

Another simulation of spaceflight stressors involves confining small numbers of people in closed environments for long periods. Early in one 90-day trial, an unexpected conflict arose among the subjects revolving around competition for the informal leadership role. This situation was aggravated by a conflict between the subjects and service personnel. Immunological analysis revealed a clear association between stress and reduction in NK-cell cytotoxicity. The largest decrease took place in the subject who was most involved in the conflicts. The group mean T-cell responsiveness to PHA was unchanged, although one subject who had a high initial RNA synthesis rate showed a significant decrease beginning on the 20th test day. The subject with the most pronounced stress response showed a considerable decline in T-helper cell function. Nonspecific suppressor activity remained within normal limits at all test sessions.

In another study, three subjects lived in a closed environment for 60 days. Five 5-day visits by groups of two or three people greatly strained the living and working conditions of the core group. Additional stresses were imposed by activating loud sirens when subjects performed tasks incorrectly or did not respond to warning signals about a life-support system malfunction. The crew was stressed further by being deprived of sleep for 3 consecutive days. Not surprisingly, group performance deteriorated and conflicts ensued. The investigators reported diminished T-suppressor cell activity, moderate suppression of NK-cell activity and T-helper cell function, and occasional decreases in PHA responsiveness. Although the above results are not completely consistent, they are a useful addition to the analysis of spaceflight effects.[6]

III. Immunological Studies of Animals Aboard Biosatellites

Immune function in rats has been tested extensively in experiments on several Soviet biosatellites. Rats flown on Kosmos-605 for 22.5 days had significant involution of both thymus and spleen after flight.[1,44] Experiments on Kosmos-782[45] assessed spleen-cell responses to nonspecific mitogens and antigen from *Listeria monocytogenes* before and after flight. Splenocytes were obtained from the flight group of rats (killed 9 to 11 days after landing) and three control groups, the first (synchronous control) suspended by their tails in a ground-based mock-up of the biosatellite, and the second and third groups being either immunized or nonimmunized rats housed in a vivarium. Splenocytes of flight rats responded well to nonspecific mitogens (PHA, concanavalin, purified tuberculin) and the bacterial antigen. Decreased lymphocyte response in rats in the synchronous group (relative to the flight and vivarium groups) was interpreted as meaning that stress on Earth inhibited immune response more than similar stress in microgravity.

Soviet/Russian and American scientists studied several aspects of cellular immunity and humoral mediators in rats flown for 7–14 days on Kosmos-1667, -1887, and -2044 missions.[45-48] T-cell proliferation was diminished after Kosmos-2044 relative to before launch, but T-suppressor function was suppressed. Splenocyte proliferation in response to recombinant IL-2 stimu-

lation was substantially suppressed after a 13-day flight on Kosmos-1887. Increases in the bone marrow T-cell population after Kosmos-1667, detected by indirect immunofluorescence, were highly selective.[49] We interpret this finding as reflecting an acute stress reaction, as manifested by suppression of T-cell redistribution from the blood and lymphoid tissue to the bone marrow.

Cytofluorimetric analyses after Kosmos-1887 and -2044 revealed shifts in immunocompetent cell subpopulations associated with spaceflight. Numbers of $w3/13^+$ cells (pan-T population) were greater after flight, confirming the migration of T cells from the bloodstream to the bone marrow. Also greater after flight were numbers of activated T cells with affinity for IL-2 ($OX\text{-}39^+$), T-helper cells ($w3/25^+$), and NK cells (asialo-$GM\text{-}1^+$). Numbers of T-helper cells in the bone marrow were higher after both flights than before. Numbers of T-helper and T-suppressor cells in the spleen were greater after flight than before, but high numbers of T cells with an affinity for IL-2 were observed only after Kosmos-1887. Bone-marrow sensitivity to monocyte/macrophage colony-stimulating factor (CSF-M) was reduced after Kosmos-1887, and its sensitivity to granulocyte/monocyte colony-stimulating factor (GM-CSF) was reduced after Kosmos-2044.[47] These compounds regulate the differentiation of specific bone-marrow cell populations; suppression of the ability to respond to them is a serious compromise of primary immunity.

NK-cell activity in spleen and bone marrow decreased significantly in rats after 7- and 14-day flights on Kosmos-1667 and -2044.[50] NK-cell function was suppressed in the flight group, and to a lesser extent in the synchronous group. The numbers of splenocytes binding K-562 target cells declined in both flight rats (Kosmos-1667,-1887,-2044) and tail-suspended rats in the synchronous control group, but did not change in the vivarium group. Moreover, changes in cytotoxicity were apparent from analysis of newly formed conjugates. Spaceflight also was associated with suppression of tumor necrosis factor alpha (TNF-α), which is produced by NK cells and macrophages. The similarities between the flight group and synchronous-control groups support the contention that stress has a substantial effect on NK-cell activity.

Humoral mediators produced by rat splenocytes and bone marrow cells also were of interest because of their roles in proliferation and differentiation of cells in the hemopoietic, bone, and nervous systems. IFN-γ production by splenocytes was shown to be reduced after the U.S. Spacelab-3 mission[51] as well as the Kosmos-1661 and -1187 missions; IL-3 production was unchanged after SLS-3.[51] IL-2 produced in response to in vitro splenocyte activation with CTLL cells or lymphoblasts was diminished markedly in animals flown on Kosmos-1667 and -1887 relative to ground controls. IFN-α and OAF activity in splenocyte-activated supernatants were no different after flight from before, but IL-1 production was enhanced. In sum, then, the flight conditions that caused a decrease in IL-2 and IFN-γ production did not affect the production of IL-3, IFN-α, or OAF, but enhanced that of IL-1 by splenocytes. By contrast, in ground-based studies, suspending rats by their tails for 1 to 2 weeks sharply reduced the production of IFN-α, IFN-β, and IFN-γ.[77] However, IFN production in rats restrained horizontally was no different than before the restraint period. Moreover, the animals suspended in a head-down position had less resistance to the encephalomyocarditis virus.[51]

Spaceflight seemed to have different effects on lymph-node cells than on splenocytes: The 14-day Kosmos-2044 mission did not seem to affect lymphocyte activation in lymph nodes, but T cells retained their capacity to be activated by ConA and PHA; B cells retained their capacity to proliferate in the presence of *E. coli* lipopolysaccharide; and production of IL-2 and IL-1 was normal.[52] These differences between splenocytes and lymph-node cells emphasize the need for more detailed study of the effect(s) of spaceflight on stress hormones and other endogenous substances with regard to the immune system.

IV. Spaceflight and Immune-Cell Cultures

Although studies such as those described above provide useful information on immune function at the organismal level, many questions remain as to how weightlessness affects immune-cell function at or below the level of the cell. However, several in vitro tests have been flown to address aspects of this broad issue. One example, the Russian-Hungarian "Interferon" experiment flown in 1980 and 1981 on Salyut-6, assessed the ability of isolated human lymphocytes to produce IFN in weightlessness.[53,54] IFN was produced both more quickly and to a greater degree by the on-board lymphocyte suspension than by a control culture on Earth: IFN level in test tubes was 4 to 8 times higher in space lymphocytes than in ground lymphocytes in the first experiment, and 2 to 4 times higher in the second.[54] All of the tested inductors (e.g., poly I:C, poly G:C, PPD, Newcasle disease virus) were effective. These data, in combination with analogous results from cosmonauts who flew at the same times, suggested that humoral factors inhibited IFN production by cells.

In another series of experiments conducted by Cogoli and colleagues,[55] ConA activation of lymphocytes aboard Spacelab-1 and Spacelab D-1 was only 3% of the preflight baseline. At the same time, cell cultures that were centrifuged in space showed some signs of activation (i.e., ^{3}H-thymidine uptake), although to a lesser extent than before launch. Analogous results were found in other experiments by the same team using whole blood obtained from four astronauts before, during, and after the flight.[56,57] Another Spacelab D-1 experiment involved autoradiography and electron microscopy of donor lymphocyte cultures; although cultures centrifuged on board showed normal activation, no activation was present in the weightless culture.

The Russian-French "Immunocytos" experiment was designed to study mechanisms of T-cell and monocyte activation in space,[58,59] and was flown on biosatellite Kosmos-2044. Jurkat cells were used to stimulate lymphocytes and THP-1

cells to stimulate monocytes, and anti-CD3 monoclonal antibodies, calcium ionophore A 23187, and phorbol myristate acetate (PMA) were used as activators. These compounds were kept in glass ampules and added to the cell suspensions 5 hours after the biosatellite began its Earth orbit, after which the cells were incubated at 37°C for 12 hours. A synchronous ground-based control culture was begun 4 hours after the launch. IL-1 and IL-2 production were measured in supernatants using immunoenzyme assay (sandwich method, ELISA); IL-1 activity was also measured in mouse thymocytes and IL-2 in 4-day-old PHA blast cells.

Jurkat cells activated by anti-CD3 antibodies in the presence of THP-1 cells produced the same amount of IL-2 in weightlessness as on Earth. In contrast, activation with calcium ionophore or PMA produced large amounts of IL-2 in the ground tests but not the flight tests. Supernatants of mixed THP-1 and Jurkat cell cultures stimulated by anti-CD3 antibodies contained comparable amounts of IL-1 in the flight and ground samples. However, IL-1 production greatly inhibited the flight samples when THP-1 cells were stimulated by PMA. IL-1 activity was suppressed in Jurkat and THP-1 flight cell cultures stimulated by anti-CD3 antibodies.

These results shed new light on the mechanisms of human T-cell activation in weightlessness. Interleukin production was suppressed in flight cultures once cells were stimulated by soluble factors, in particular PMA. Phorbol esters are hydrophobic substances that must interact with the cell membrane in order to be bound by receptors (which have been identified as protein kinase C). Activation of the latter is accompanied by translocation of the enzyme on the cell membrane. One stage of this process may be inhibited in weightlessness. Thus, weightlessness seems to delay the synthesis of mediators when lymphocytes and monocytes are activated by soluble hydrophobic substances. However, the lack of effect on IL-2 production suggests that spaceflight factors did not affect intercellular interactions. An additional experiment was performed by French scientists in parabolic flight, in which Jurkat cells were used with phorbol-12,13 dibutyrate, a labeled phorbol ester.[60] This phorbol compound was found to bind T cells specifically during the free-fall phases of parabolic flight, implying that PMA uptake in the cell membrane was not impaired in brief simulated weightlessness.

Many questions remain as to how a single somatic cell that is integrated in a multicellular organism can recognize gravity, and how elimination of the "gravity recognition" function in weightlessness affects isolated cells. Both cell recognition and cell locomotion undoubtedly play important roles in this process. Leukocytes (i.e., nucleated cells of the myeloid and mononuclear-phagocytic systems) have highly developed locomotor apparati in the form of cytoskeletal microtubules and microfilaments. Some lymphocytes recirculate continuously in the blood and lymphatic systems, and thus are subject to strong hydrodynamic effects. Lymphocytes also participate in cell recognition, homing, and migration, all of which involve mechanical effects of the locomotion apparatus and the plasma membrane, none of which were addressed in the experiments described above.

Contacts between lymphocytes and other cells trigger numerous events. For example, interactions between an NK cell and a target cell involve intensive participation of the intracellular apparatus (particle movement and exocytosis, shifts in orientation of the Golgi complex, etc.). Since recycling is associated with intensive and goal-directed locomotion, these processes could be expected to be impeded in weightlessness.

If cells recognize gravity indirectly by responding to strain on the cytoskeleton (which could be accompanied by deformation of the membrane of the sensor cell), then lack of contact between the lymphocytes and substrates and other cells and loss of the locomotor function in weightlessness could create a condition in the lymphocyte culture that resembles that of osteocytes in intact bone in space.

These experiments on isolated immunocompetent cells represent dramatic progress. The progression from early, relatively simple studies to complex experiments using modern immunological tools has provided much insight into cellular and molecular processes in weightlessness. However, many problems remain to be resolved.

V. Osteoclast-Activating Factor and Calcium Metabolism in Bones

Prolonged exposure to weightlessness leads to loss of minerals, particularly calcium, from the skeleton. This effect, regardless of its ultimate cause, could be the limiting factor in safe extension of the duration of human flight.[61–63]

Bone-metabolism studies in the Gemini-7 and Skylab missions revealed heightened catabolism in the musculoskeletal system: Calcium and phosphorus balances became negative, and bone mineral content declined.[63] Calcium continued to be lost throughout the flight, approaching the levels typical of prolonged hypokinesia on Earth.[64] Grigoriev and others have postulated that long-term flight leads to increased renal excretion of osmotically active substances, particularly calcium and potassium. The greatest amounts of electrolytes excreted to date were those recorded at the end of the first month of a 175-day flight.[65] Hypokinesia also inhibits endochondral and periosteal osteogenesis,[66] causing osteoporosis in the spongy tissue and perilacunar osteolysis in the compact bone.

Impairment of osteogenesis was assessed at length in mature and young growing rats on Kosmos-782, -936, and -1129.[67–69] Supporting bones were found to lose strength; periosteal growth was inhibited, but the growth rate of endosteal tissue was normal. These investigators attributed the onset of osteoporosis under these conditions to interference with the osteoblast repair processes. Prolonged immobilization of humans, as in bed rest, leads to activation of osteoclasts that are responsible for bone resorption, and to reductions in osteoblast activity.[70]

Numerous data exist to support the concept that the immune system affects regulatory processes determining bone mineral content, growth, and remodeling. For example, introducing allogeneic lymphocytes into young animals stops

growth and bone-mass increase[71]; thymectomy in neonatal mice terminates their growth. Thus, some relationship exists between regulation of bone-calcium metabolism and osteoclast function on the one hand, and the primary immune organs (thymus and bone marrow) on the other. Disorders of the thymus (and consequent T-cell malfunctions) play a significant role in impairment of bone mineralization.[72] Thymic size and weight are both less than normal in rodents having congenital impairment of bone-mineral metabolism; not surprisingly, these animals have numerous immune-system dyfunctions as well. Another study involving prolonged administration of dichloromethylene diphosphonate to 3-day-old rats showed increases in bone-calcium resorption that were correlated with thymus and T-cell impairments.

Of the many humoral factors involved in regulating bone resorption and osteogenesis, osteoclast-activating factor (OAF), which was discovered through the study of cell-mediated immune response to chronic inflammatory processes accompanied by osteoporosis,[73] is of particular interest. OAF is produced in vivo by peripheral-blood leukocytes in response to PHA and other mitogens or in the presence of an antigen to which they have been sensitized.[74] The effect of OAF on bone in vitro is comparable to that of parathyroid hormone. Studies of bone resorption in the presence of chronic inflammation or in newly generated bone tissue have revealed that bone destruction requires the presence of OAF and prostaglandins, both locally acting substances. We propose that OAF mediates bone resorption in weightlessness and hypokinesia.[6,23] We further postulate that OAF acts in concert with IL-1 and other factors to maintain continuous physiological equilibrium between osteoblasts and osteoclasts. Prolonged hypokinesia or spaceflight produces changes at the beginning and end of the chain of events governing bone tissue homeostasis. As a result, the activity of several immunocompetent cell populations changes and calcium simultaneously is lost from bone. We believe these processes to be interrelated such that impairment of bone homeostasis could result from changes in the immune system.

Spaceflight, regardless of duration, is inextricably associated with forms of stress other than weightlessness. Chronic stress during long-term flights seems to result mostly from physiological adaptation of the human body to the unfamiliar environment of space (limited motor activity, altered afferent impulses from postural and tonic muscles, the absence of the effects of a supporting surface, and diminished or absent hydrostatic pressure produced by shifts of blood and body fluids). Migration of certain immunocompetent cells into the bone marrow and impairment of cell migration may become normal once the body has adapted to weightlessness. However, some periods that confer acute stress also are inevitable in flight (e.g., EVAs, unexpected repairs, or emergencies). Hence, T cells could migrate frequently into the bone marrow during flight.

How do large numbers of mature T cells (and other cells) remaining in the bone marrow for prolonged periods affect bone homeostasis? They could elevate local concentrations of OAF or other cytokines involved in bone resorption. A feedback mechanism may exist within the system governing cell differentiation that determines the size of the areas in spongy bone in which stem cells are generated. Experiments of induced osteoporosis have demonstrated the rapidity with which changes in blood cells can reduce the number of medullary cavities filled with unresorbed endochondral bone trabeculi. These findings also suggest the existence of factor(s) (e.g., OAF, IL-1, or others) that could regulate spongy-bone structure and metabolism. New studies, designed specifically to address these questions, are needed to confirm these suppositions.

We have begun to study OAF production by peripheral-blood lymphocytes from humans undergoing head-down bed rest. OAF activity was measured in supernatants of mitogen-stimulated lymphocyte cultures, which had been added to cultures of long tubular bone, labeled with ^{45}Ca, from 17-day-old mouse embryos. OAF resorption activity was assessed by measuring ^{45}Ca released from the bone tissue.[75,76] Estimates of the normal range of the resorption index (RI) were generated from blood samples drawn from healthy volunteers that had normal work-rest schedules. The nine bed-rest subjects were all found to have increased RI, and the extent of the increase was directly related to the duration of bed rest[76] (Fig. 10). Two of the eight subjects examined late during the bed-rest period had periodontitis, and biochemical tests conducted throughout the bed-rest period showed consistent increases in the excretion of Ca and its ionized fraction. We conclude that elevated OAF production is one manifestation of the changes induced by head-down bed rest. A linear relationship was established between the duration of treatment and the probability of OAF effects, at least during the first eight months of a 350-day study. OAF activity also was elevated in three of eight cosmonauts after flights on Salyut-7 and Mir. Much additional research is needed in order to supplement these intriguing results.

VI. Conclusions and Prospects for Future Research

The findings described here were derived from research with human and animal subjects before and after spaceflight, healthy subjects undergoing weightlessness simulation, and isolated human blood cells exposed to weightlessness. The factors that affect immunity during spaceflight can be ranked, in descending order of importance, as follows: weightlessness; stress; hypokinesia; confinement in closed environments; exogenous and endogenous microflora and allergens; and space radiation. The syndromes arising from these factors that are of particular importance are:

- immune impairment arising from exposure to weightlessness and hypokinesia, mediated by shifts in calcium metabolism, neuroendocrine functions, and immunocyte migration;
- a "stress syndrome" that triggers changes in the hormonal and neural regulatory processes within the microcom-

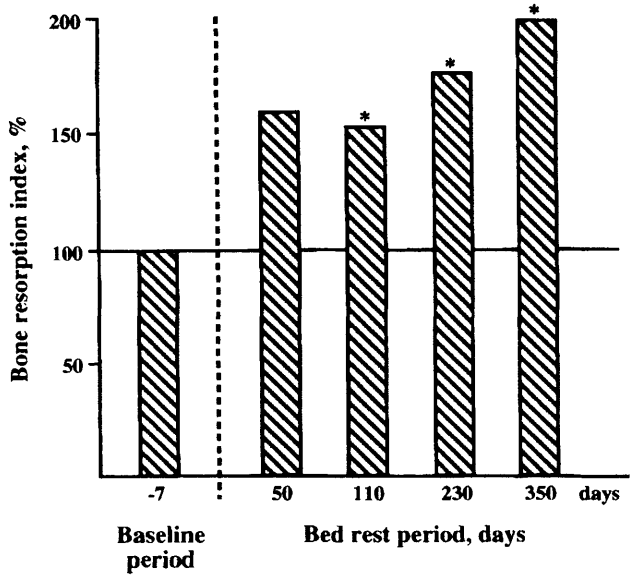

Fig. 10 Production of osteoclast-activating factor by human lymphocytes from subjects undergoing up to 350 days of head-down bed rest.

partments of the immune system, which in turn affect neuroendocrine regulation (perturbing the homeostasis of the neurohumoral-immune system); and

- changes in the immune system related to living in closed environments, and the effects of varying gaseous composition and contaminants within those environments.

The relationship between immunocompetence of crew members and the status of exogenous and endogenous microflora, as well as the constant presence of allergens in the atmosphere, constitute a separate issue that is addressed elsewhere in Volume II of this series. However, sensitization to various allergens during long-term spaceflights is a logical outgrowth of some of the results discussed here, and underscores the importance of increasing research efforts on these issues.

Distinguishing among the effects arising from weightlessness vs stress per se is difficult given the ubiquitous nature of stress, which can arise not only from emotional factors but also, in a more mechanical sense, from the lack of musculoskeletal loading. Comparing results from brief vs long flights may facilitate understanding of the differences and interrelationships between these two factors. For example, Taylor and Dardano[11,12] proposed that suppressed human lymphocyte responsiveness to PHA noted after Space Shuttle missions could have been caused indirectly by the stresses involved in trying to maintain postural equilibrium in a weightless environment. On the other hand, PHA responsiveness in crew members after 7- or 8-day missions aboard Salyut-6, Salyut-7, and Mir was consistently normal, suggesting that brief Space Shuttle missions, or perhaps the stresses associated with landing, produced greater stress effects.

Undoubtedly, the effects of weightlessness, emotional stress, and other long-term spaceflight factors on immunocytes are not direct, but are mediated by the neurohumoral system. Talas and Konstantinova[53,54] have demonstrated an increase in the ability of isolated lymphocytes exposed to weightlessness aboard a spacecraft to produce IFN. However, IFN production by blood lymphocytes from cosmonauts was decreased after landing, which probably reflects the influence of neurohumoral factors in vivo.

Cogoli and others[55–57] proposed that weightlessness suppresses mitogen-stimulated lymphocyte proliferation, whereas hypergravity activates it. Our experiment on Kosmos-1667[49] demonstrated for the first time substantial increases in T-cell counts in the bone marrow of rats. Since T-cell migration into the bone marrow indicates physiological stress, this finding complements the above analysis of the effects of weightlessness and stress on the immune system of animals and humans. This observation allows us to treat neuroendocrine shifts in the organism as primary and dominant and changes in the immune system as subordinate to them.

Functions of the immune and hemopoietic systems are tightly bound to the condition of the human musculoskeletal system, particularly bone. This circumstance takes on particular significance with regard to space-related osteoporosis. Long-term hypokinesia seems to be accompanied by enhanced OAF production, although some subjects exhibited activation of lymphocytes that are effectors of DTH. Similar results have been observed in space crews after flight. On the basis of these findings, we have suggested several hypotheses requiring experimental verification. First, DTH effectors could be a lymphocyte subpopulation synthesizing OAF, with their activity increasing in spaceflight. Second, activation of T-cell effectors that mediate DTH could be related, under extreme conditions, to changes in suppressor T-cell activity. This would shift the balance between T-suppressor and DTH T-effector activity and elevated OAF production. Third, the increased T-cell population in the bone marrow might elevate local production of OAF. Finally, since OAF is a product of hemopoietic lymphocyte cells, OAF could regulate the size of sites in spongy bone tissue responsible for hemopoiesis.

The critical association between the immune and hemopoietic systems on the one hand, and bone metabolism on the other, has already been established. OAF is one of the effector and regulatory molecules of this system. We believe that our hypothesis about the special role of the immune system in the impairment of bone metabolism, specifically calcium metabolism during exposure to weightlessness, has been confirmed experimentally. Now, we must learn how the above factors are affected by weightlessness and hypokinesia. This research will facilitate development of countermeasures that will ameliorate bone-calcium loss in flight.

In conclusion, the past two decades of research have revealed that spaceflight factors suppress the immune system of humans. Immune-system adaptation to entirely new conditions in evolutionary terms is of great interest to theoretical biologists. At the same time, the practical importance of these findings is indisputible, since these deviations indicate secondary immunodeficiencies that increase the risk of diseases in

spaceflight. Future studies of the immune system thus should be focused on developing and implementing effective means of correcting altered responses of the human immune system in spaceflight. Although several antiallergic and antiviral drugs have already been introduced, major developments are still ahead.

Several specific issues remain to be addressed, in particular the relationships among the nervous, endocrine, and immune systems. Such an investigation would target hormones, neuropeptides, and their receptors on the surfaces of different cells under normal and extreme conditions. This approach will facilitate the development of countermeasures (including drugs) acting on the immune system both directly and through mediation of neuroendocrine mechanisms. Immunological interactions also must be addressed at the cellular level, e.g., the interaction of lymphocyte subpopulations participating in immune responses with products of altered bacterial and viral autoflora (lipopolysaccharides, enterotoxins, etc.). In-depth studies of the human immune system in flight are within reach if automated, state-of-the-art technology can replace conventional methods of immunological research in space.

Another promising direction for future research is the development of means to regulate bone-calcium metabolism with the immune system as a mediator. Investigations of both direct (through OAF, IL-1, etc.) and indirect feedback mechanisms (through calcium) between the immune system and bone are still more urgent. Such studies would be complemented by identifying and testing new biologically active substances that can correct immune-system deviations. Solving these and other problems yet to be discovered will require the use of novel methods and the latest discoveries in molecular and cell immunology by experts from around the world.

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